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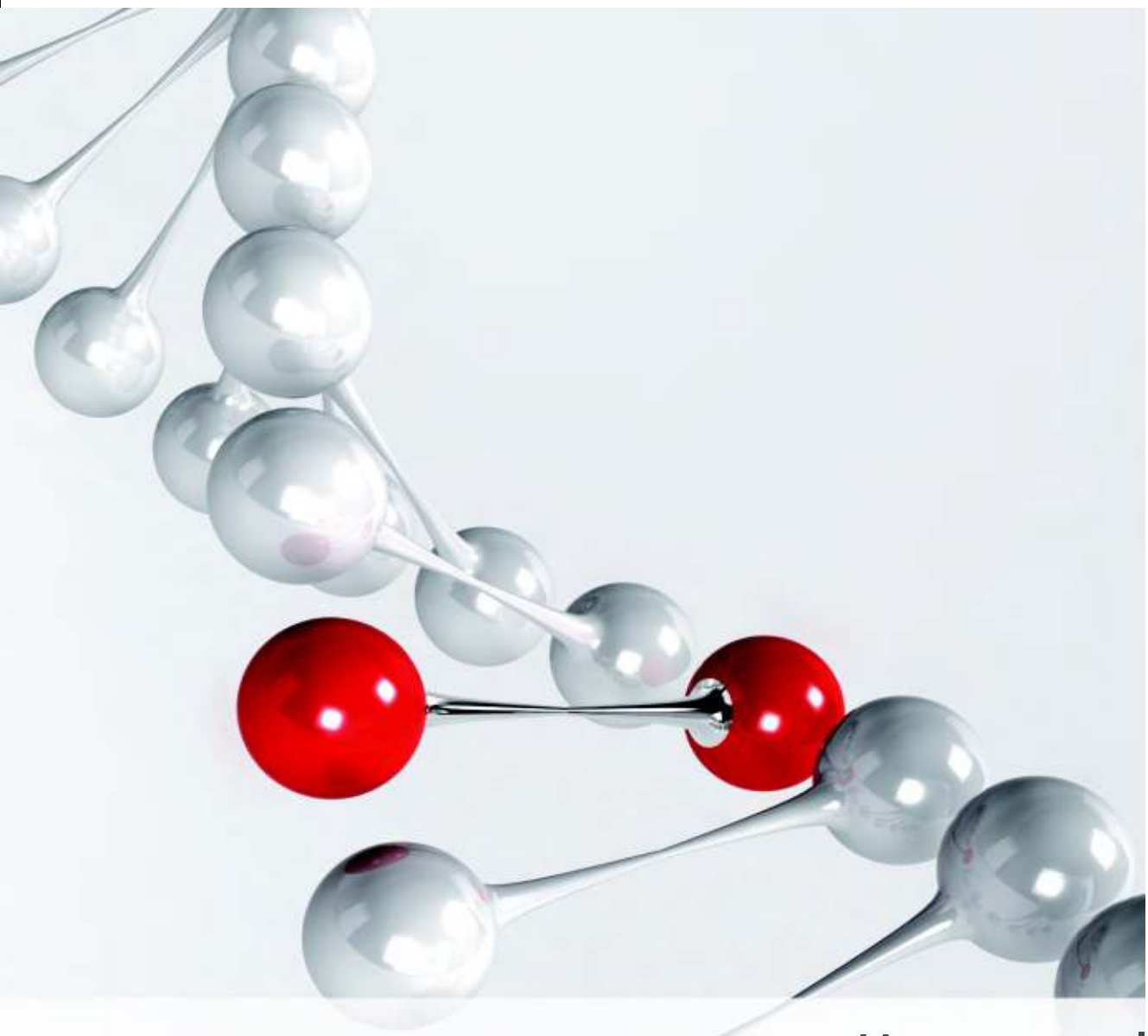

User manual

InviMag® Blood DNA Maxi Kit/ IG

for use on the InviGenius®, STRATEC Molecular GmbH

for automated purification of DNA from 2 ml whole blood samples with magnetic beads

REF 2431320x00 STRATEC Molecular GmbH, D-13125 Berlin

Instructions for InviMag® Blood DNA Maxi Kit /IG

The **InviMag® Blood DNA Maxi Kit /IG** combines the advantages of the innovative Invisorb® technology with easy handling of magnetic particles in combination with the InviGenius® robotic platform for an efficient and reliable isolation of nucleic acids with a high purity.

The **InviMag® Blood DNA Maxi Kit /IG** is the ideal tool for walk-away automated isolation and purification of highly pure total (genomic and mitochondrial) DNA from 2 ml whole blood samples (stabilized with EDTA or citrate but ***not*** heparin). The kit is designed for use on the InviGenius® workstation. The interplay of the DNA extraction and purification chemistry provided by the **InviMag® Blood DNA Maxi Kit /IG** was intensely tested and validated.

The DNA-binding magnetic particles are characterized by a high surface area, uniform size distribution, and good suspension stability and therefore are highly suitable for high-throughput processing.

Due to the high purity, the isolated DNA is ready-to-use in a broad panel of downstream applications or can alternatively be stored at -20°C for subsequent use.

The kit is neither validated for the isolation of genomic DNA from cell cultures, tissues, blood cards, dried blood stains nor urine. The application of the kit for isolation and purification of viral DNA has neither been tested nor evaluated.

For research use only!

Trademarks: InviMag®, Invisorb®, InviGenius®. Registered marks, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

The Invisorb® technology is covered by patents and patent applications: US 6,110363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

InviMag®, Invisorb®, InviGenius® are registered trademarks of STRATEC Biomedical AG.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

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Kit contents of the InviMag® Blood DNA Maxi Kit /IG

Store the **MAP Solution B** at 2 - 8°C!
Store lyophilized **Proteinase K** at 2 - 8°C! Dissolved **Proteinase K** must be stored at –20 °C!
Store all other kit components at room temperature (RT)!

Component		8 x 12 preps
Catalogue No.	2431320100	
Lysis Buffer HLT	4 x 80 ml	2 runs* per bottle
Proteinase K/ IG	8 x 80 mg (8 x 2.4 ml working solution)	1 vial per run*
MAP Solution B/ IG	8 x 3.3 ml	1 vial per run*
Binding Solution (fill with 99.7% Isopropanol)	4 x empty bottles (final volume 4x 110 ml)	2 runs* per bottle
Wash Buffer	4 x 36 ml (final volume 4x 120 ml)	2 runs* per bottle
Elution Buffer M	4 x 60 ml	2 runs* per bottle
Incubation Plate D	8	1 run* per plate
Working Plate A	8	1 run* per plate
Elution Plate E	1	8 runs* per plate
Sheath Box	2 x 48 sheaths racks	4 runs* per rack
Initial steps	Add 110 ml **Isopropanol** (molecular biologic grade, undenaturated) to each empty bottle labeled with “Binding Solution”. Keep unused bottles firmly closed Resuspend a lyophillized **Proteinase K** tube with 2.4 ml dd-H_2O and mix thoroughly! Add 36 ml of 96-100% ethanol to the bottle **Wash Buffer**, mix thoroughly and always keep unused bottles firmly closed!	

* One run is defined as a run using 12 samples

Please keep in mind, that partially used bottles may be used in other runs. The InviGenius tracks the sample numbers and runs that were extracted with each bottle. MAP Solution B and Proteinase K may be only used in two runs for quality mixing.

Symbols

Manufacturer

Lot number

Catalogue number

Date of manufacture

Expiry date

Consult operating instructions

Temperature limitation

Do not reuse

Storage

All buffers and kit contents of the **InviMag® Blood DNA Maxi Kit /IG**, except **Proteinase K** and **MAP Solution B** (magnetic beads) should be stored at room temperature* and are stable for at least 12 months at this condition.

Proteinase K /IG: Lyophilized Proteinase K should be stored at 2-8°C. Dissolved Proteinase K must be stored at -20°C.

MAP Solution B /IG: The magnetic beads should be stored at 2-8°C.

Wash Buffer and Binding Buffer: Wash Buffer and Binding Buffer charged with either ethanol or isopropanol should be stored at room temperature and must be appropriately sealed. If there are any precipitates visible within the provided solutions, solve them by carefully warming up to 30°C.

Room temperature (RT) is defined as a range from 15-30°C

Quality Control and product warranty

STRATEC Molecular warrants the correct function of the **InviMag® Blood DNA Maxi Kit/ IG** for applications as described in this manual. Purchaser must determine the suitability of the product for its particular use. Should any product fail to perform the applications as described in the manual, STRATEC Molecular will check the lot and if STRATEC Molecular investigates a problem in the lot, the product will be replaced free of charge.
STRATEC Molecular reserves the right to change, alter, or modify any product to enhance its performance and design at any time.
In accordance with STRATEC Molecular's ISO 9001-2000 and ISO EN 13485 certified Quality Management System the performance of all components of the **InviMag® Blood DNA Maxi Kit/ IG** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.
If you have any questions or problems regarding any aspects of **InviMag® Blood DNA Maxi Kit/ IG** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

For technical support or further information please contact:
from Germany: +49-(0)30-9489-2901/ 2910
from abroad: +49-(0)30-9489-2903/ 2907 or contact your local distributor.

Intended use

The **InviMag® Blood DNA Maxi Kit /IG** is designed for fully automated extraction and purification of genomic DNA from up to 2 ml whole blood in the range of 1 – 12 blood samples using the InviGenius® robotic platform.

The nucleic acid isolation protocol is suitable for routinely walk-away automated preparation of DNA from fresh or frozen blood samples. For reproducible and high yields an appropriate sample storage is essential (see “Sampling and storage of the starting material”, page 8).

Common blood collection tubes (not provided) and anticoagulants (EDTA and citrate, but ***not*** heparin) can be used. All utilities (reagents and plastics, besides filter tips) required for preparation of DNA from blood are provided by the **InviMag® Blood DNA Maxi Kit /IG.**

THE PRODUCT IS INTENDED FOR USE BY PROFESSIONALS SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of DNA/ RNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings. To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

Product use limitation

The kit is only validated for the extraction of human whole blood samples. The isolation of DNA from other sources like stool samples, tissues, bacteria, fungi or viruses was neither tested nor validated. No guarantee in operability is issued if other samples than whole blood are used.

The included chemicals are only useable once.

Differing of starting material may lead to inoperability. Therefore, neither a warranty nor guarantee in this case will be given, implied or expressed.

The user is responsible to validate the performance of the STRATEC Molecular product for any particular use. STRATEC Molecular does not provide validations of performance characteristics of the product with respect to specific applications. STRATEC Molecular products may be used e.g.in clinical diagnostic laboratory systems conditioned upon the complete diagnostic system of the laboratory the laboratory has been validated pursuant to CLIA’ 88 regulations in the U.S. or equivalents in other countries.

All products sold by STRATEC Molecular are subject to extensive quality control procedures (according to ISO 9001-2000 and ISO EN 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon detection thereof.

The chemicals and the plastics are for laboratory use only. They must be stored in the laboratory and must not be used for other purposes than intended.

The product with its contents is not suitable for consumption.

Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles!

Avoid skin contact! Adhere to the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at www.stratec.com for each STRATEC Molecular Product and its components. If buffer bottles are damaged or leaking, **WEAR GLOVES AND PROTECTIVE GOGGLES** when discarding the bottles in order to avoid any injuries.

STRATEC Molecular has not tested the waste generated by the **InviMag® Blood DNA Maxi Kit /IG** procedures for residual infectious materials. Contaminations of the waste with residual infectious materials is highly unlikely but cannot be excluded completely. Therefore, all generated waste has to be considered infectious and should be handled and discarded accordingly to local safety regulations.

Below European Community risk and safety phrases for the components of the **InviMag® Blood DNA Maxi Kit /IG** are listed.

Lysis Buffer HLT, Wash Buffer HLT

warning

contains guanidine-hydrochloride;

H302-315-319, P280-305+351+338

Proteinase K

danger

H315-319-334-335 P280-305-351-338-310-405

H315:	Causes skin irritation.
H319:	Causes serious eye irritation.
H334:	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335:	May cause respiratory irritation.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338:	If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses and continue rinsing.
P310:	Immediately call a POISON CENTER or doctor/physician.
P405:	Store locked up.

Emergency medical information can be obtained 24 hours a day from infotrac:

outside of USA: 1 – 352 – 323 – 3500
inside of USA: 1 – 800 – 535 – 5053

Product characteristics of the InviMag® Blood DNA Maxi Kit /IG

The **InviMag® Blood DNA Maxi Kit /IG** is the ideal tool for an efficient and fully automated DNA extraction and purification from fresh or frozen whole blood samples using magnetic beads in combination with the InviGenius® robotic platform.

Starting Material	Yield	Time for preparation	Ratio
2 ml fresh or frozen whole blood (stabilized with EDTA or citrate but not heparin)	20 - 50 µg, depending on the blood sample (transport, storage, age and source)	about 170 min (12 samples)	A_{260}:A_{280}: 1.7-1.9

The DNA isolation process is based on the interaction of nucleic acids with silica coated magnetic particles at adapted buffer conditions. The InviGenius® instrument will automatically perform the whole DNA purification procedure, including sample and reagent distribution, without any user intervention, except the initial loading of the system. This allows safe handling of potentially infectious samples whereas cross-contaminations and reagent cross-overs are effectively eliminated by the automated purification process. The use of unique bar codes for samples and reagents prevents unwanted transpositions.

The InviGenius® instrument uses magnetic rods to transport the DNA-binding magnetic particles through the various extraction phases such as binding, washing and elution. The volume of reagents required for DNA isolation is reduced to a minimum. Eliminating the direct liquid handling and increasing the automation level results in a fast, reliable and robust technique.

After a sample specific lysis using **Lysis Buffer HLT** and **Proteinase K**, optimal binding conditions are adjusted by addition of **Binding Solution.** The genomic DNA binds to the simultaneously added magnetic particles and is separated from solution using magnetic rods which are controlled by the InviGenius® system. Subsequent to seven washing steps of the particle bound nucleic acids, pure DNA is finally eluted.

Due to the high purity, the eluted total DNA (genomic and mitochondrial) is ready-to-use in a broad panel of downstream applications:

- PCR, real-time PCR
- Restriction enzyme digestion
- HLA typing
- Southern Blotting

For the isolation of DNA from single blood samples, STRATEC Molecular offers the **Invisorb® Spin Blood Mini Kit** or for 8 – 96 samples the **Invisorb® DNA Blood Mini HTS 96 Kits** for use on a centrifuge, vacuum manifold or other robotic stations.

For further information please contact: +49 (0) 30 9489 2901, -2910 in Germany, +49 (0) 30 9489 2903, -2907 from foreign countries or ask your local distributor.

Sampling and storage of starting material

For reproducible and high yields the appropriate sample storage is essential. Yields may be varying from sample to sample depending on factors such as health of the donor, sample age, sample type, transport and storage conditions.

Blood:

Best results will be obtained with fresh whole blood samples. Mammalian blood samples (stabilized with EDTA or citrate but **not** heparin) are stable at room temperature (18-25°C) for 2-3 hours after collection. For short-term storage (up to 24 h) samples can be stored at 4-8°C. For long-term storage, freezing the samples at -20°C or -80°C is recommended. Avoid multiple thawing and freezing cycles of samples before isolating the DNA because this may lead to degradation of the DNA.

Various different primary tubes, blood collection systems (e.g. Sarstedt, Greiner) and anticoagulants (except heparin) can be used to collect blood samples for the **InviMag® Blood DNA Maxi Kit /IG** procedure.

STRATEC Molecular will not take responsibility if other sample types are used than described.

Principle and procedure

The **InviMag® Blood DNA Maxi Kit /IG** procedure comprises the following steps:

- Lysis of blood cells and protein digestion
- Binding of DNA to magnetic beads
- Washing of the bead bound DNA and elimination of ethanol
- Elution of pure DNA

After lysis, the DNA binds to the magnetic beads whereas contaminations and enzyme inhibitors are efficiently removed during the following seven washing steps. At the end, highly purified DNA is eluted.

Lysis

Samples are lysed in a Deep Well Reservoir Plate at elevated temperatures in the presence of **Lysis Buffer HLT** and **Proteinase K**.

Binding

After addition of **Binding Solution** and **MAP Solution B** (magnetic beads) to the lysate, the DNA is bound to the beads.

Removing residual contaminants

Contaminants are efficiently removed using **Wash Buffer HLT** and **Wash Buffer** while the DNA remains bound to the magnetic beads.

Elution

The DNA is finally eluted in **Elution Buffer M**. The eluted DNA is ready-to-use for different subsequent downstream applications like PCR amplification, digestion with restriction enzymes, Southern hybridizations, HLA typing, etc.

Yield and quality of genomic DNA

The amount of purified DNA using the **InviMag® Blood DNA Maxi Kit /IG** procedure from whole blood depends on the leucocytes content, sample source, transport condition, storage and age.

Typically, a volume of a 2 ml whole blood sample from a healthy individual with an elevated white blood cell content - ranging from $3x10^6$ to $1x10^7$ cells per ml - will yield at least 20 µg of genomic DNA. The overall purified DNA yield is about 20-50 µg DNA derived from 2 ml whole blood. However, if a whole blood sample is mixed with anticoagulant containing buffer solutions the overall leukocyte concentration decreases and the yield of the DNA extraction procedure is reduced slightly.

Important notes

Important points before starting a protocol

After receiving the kit, check all components for visible damage. If buffer bottles are damaged or leaking contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to "Safety information" (page 6). Do not use damaged kit components because their use may lead to poor kit performance.

- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
- Discard gloves if they become contaminated.
- Do not combine components of different kits.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of diseases from potentially infectious material, we recommend working under laminar air-flow for all steps performed outside the InviGenius® platform.
- This kit should only be used by trained personnel.

Preparing reagents and buffers

Before starting a run, equilibrate all reagents at room temperature. Where necessary, gently mix and redissolve any precipitates by incubating at 30°C. Swirl gently to avoid foaming.
Lysis Buffer HLT and **Elution Buffer M** are ready-to-use.

8 x 12 DNA-extractions
Resuspend a lyophilized **Proteinase K** tube by addition of 2.4 ml dd-H_2O, mix thoroughly and store diluted and unused **Proteinase K** at –20°C.
Add 36 ml of 96-100% ethanol to the bottle Wash Buffer II. Mix thoroughly and always keep the bottle firmly closed!
Add 110 ml of 99.7% isopropanol to the bottle **Binding Solution** and always keep the bottle firmly closed!

Reagents and equipment to be supplied by user

- Measuring cylinder (250 ml)
- Pipette tips
- Disposable gloves
- ddH_2O
- Vortexer
- 96-100% ethanol
- 99.7 % Isopropanol*

*The **InviMag® Blood DNA Maxi Kit /IG** is validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from **Carl Roth.**

*** Possible suppliers for Isopropanol:**

Carl Roth
2-Propanol
Rotipuran >99,7%, p.a., ACS, ISO
Order no. 6752

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

Sigma
2-Propanol
Order no. 59304-1L-F

Important indications

1. Minimum volume of samples in primary tubes

The procedure of the **InviMag® Blood DNA Maxi Kit /IG** has been optimized for the isolation of genomic DNA from up to 2 ml human whole blood. We advise to provide at least 2.5 ml blood per sample tube (12 mm diameter, more if a wider diameter is used) to prevent pipetting distribution errors during processing and to avoid unwanted flagged sample results due to used low sample volumes.

2. Sample volume smaller than 2 ml

For samples with a volume ≤ 2 ml, please adjust the final volume with either distilled water or 1x PBS to 2.5 ml.

3. Elution volume

The final processed elution volume is 400 µl. After the elution step, an aliquot of about 380 µl is transferred to the elution plate. Typically, 400 µl eluate will contain about 20-50 µg of purified DNA.

General overview of the InviGenius® system

Figure 1: Layout of the InviGenius® system

There are three plate positions available in the InviGenius® system which can be loaded with the corresponding plates: the incubation (A), working (B) and elution position (C).

The lysis is performed at the incubation position (A), whereas the washing and elution process is performed at the working position (B). The eluate - containing the extracted nucleic acids – will be finally transferred to the elution position (C).

There are three loading positions for disposable tip trays (D1-D3) and one position (E) for the disposable sheaths available.

The loading bay (F) is located at the very right side of the instrument. The sample rack is loaded into the far left lane whereas the reagent rack is located at the right position of the loading bay.

The moveable Magnetic Separation Head (MSH) (G) is located on top of the incubator lid (parking position). The automatic pipettor head (H) is installed above the loading bay (parking position). The disposable waste tray (I) is located behind the lower cover of the InviGenius® system.

User interaction with the InviGenius® instrument is performed by use of the touch LCD (J) located at the top front right side of the instrument.

Preparing and loading of the InviGenius® system

Preparing the reagents:

Before starting a run, prepare one vial of Proteinase K and add ethanol to the Wash Buffer and isopropanol to the Binding Solution bottle as indicated in Tab. 1 (see page 3 or page 9).

Preparing the system:

Turn on the InviGenius® system using the power switch located at the back right side of the instrument. The InviGenius® software will automatically be loaded after the system has booted up. The door of the InviGenius® system must be closed during system initialization and runs.

After booting and successful initialization of the InviGenius® system a log-in screen appears (Figure 2). Log-in with the provided user name and password.

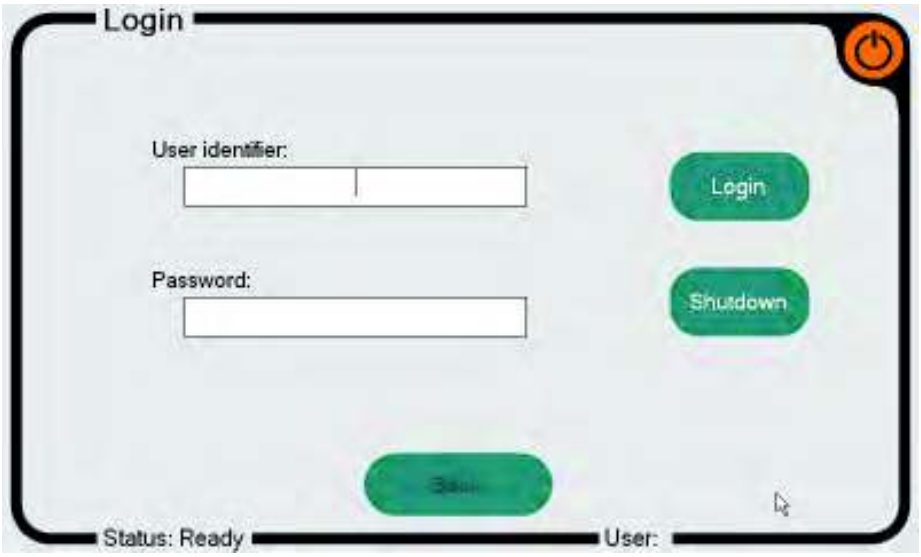

Figure 2: Log-in screen of the InviGenius® software

After logged in, the main screen of the InviGenius® software is displayed (Figure 3). Select “Loading” (A) to proceed with loading of the system or select “Processing” (B) to define and run an assay if the system has been loaded already.

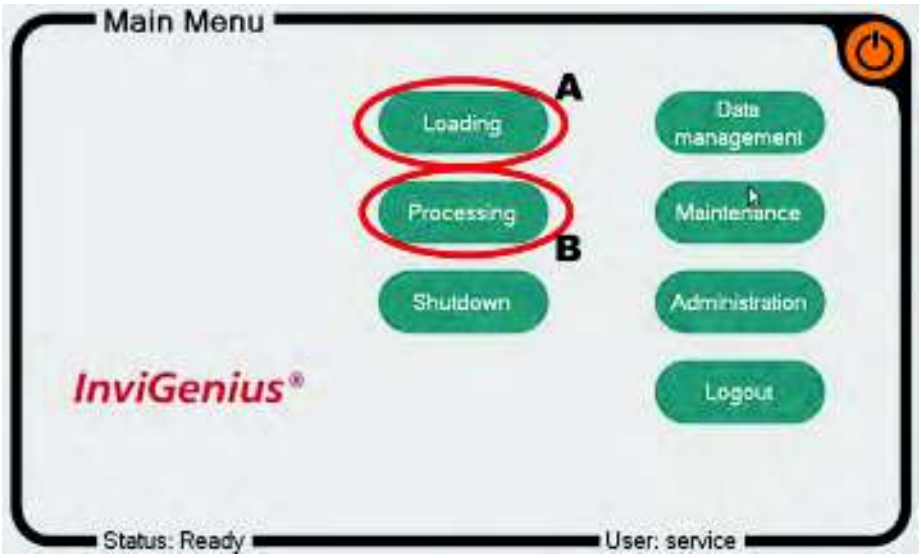

Figure 3: Main menu of the InviGenius® software

Sample Loading:

After “Loading” was selected the sample loading screen is shown. Select “Samples” (A) to proceed with the sample loading process.

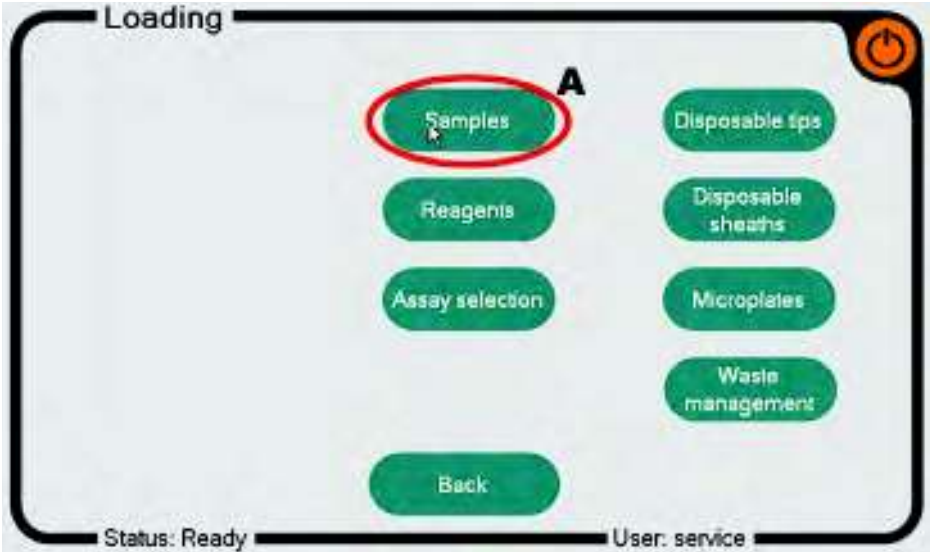

Figure 4: “Loading” submenu of the InviGenius® software

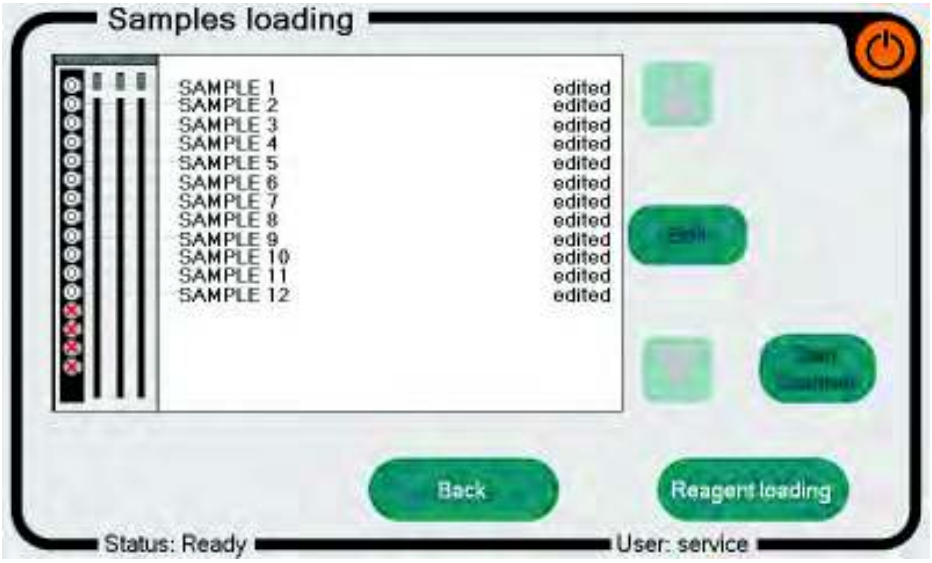

Figure 5: “Sample-loading” screen of the InviGenius® software

Prepare the sample rack with primary sample tubes that are prefilled with blood from which the DNA shall be extracted. Sample tubes are not provided within the kit. The operator must either use primary tubes provided from the blood collection, blood bank, etc. In case that no primary tubes are available, common collection tubes can be ordered at Sarstedt for example (order no. 55.476, 5 ml tubes, 75x12 mm, polystyrene).

For each reaction a total sample volume of 2 ml is processed. However, we advise that the total provided sample volume should be at least 2.5 ml to ensure stable processing. If primary tubes are used that are completely filled with blood (≥ 5 ml), please premix by inverting the tube before usage to ensure a homogenous solution. Always decap inserted sample tubes.

Keep in mind that only 12 positions of the sample rack can be processed per run due to the limited number of wells per row of the plastic ware. For correct identification of the sample tubes, bar codes (if available) must face to the bar code scanner window located at the right side of the loading bay.

After inserting the sample rack in the very left lane of the loading bay, an updated screen will show the identifiers read-out from the sample bar codes (Figure 5). In case of unsuccessful sample identification, remove the rack, check the bar code orientation and reinsert the rack slowly. If no sample barcodes is present, the corresponding sample will be flagged as “unknown sample”. In that case the sample name has to be entered/changed manually using the “Edit” button.
Keep in mind that samples marked as “unknown sample” will not be processed.

It is possible to rename barcode recognized samples too. Select the corresponding sample using the arrow buttons followed by the “Edit” button and enter the desired sample name.

After a short time period (about 5 min) the bar code scanner will be deactivated. In that case the operator has to restart the scanner by pressing the “START SCANNER” button.

After all samples have been loaded, recognized and/or renamed, proceed with reagent loading by selecting “Reagent loading” on the bottom right hand side of this screen.

Reagent Loading:

The reagent loading process is analogous to the sample loading procedure.

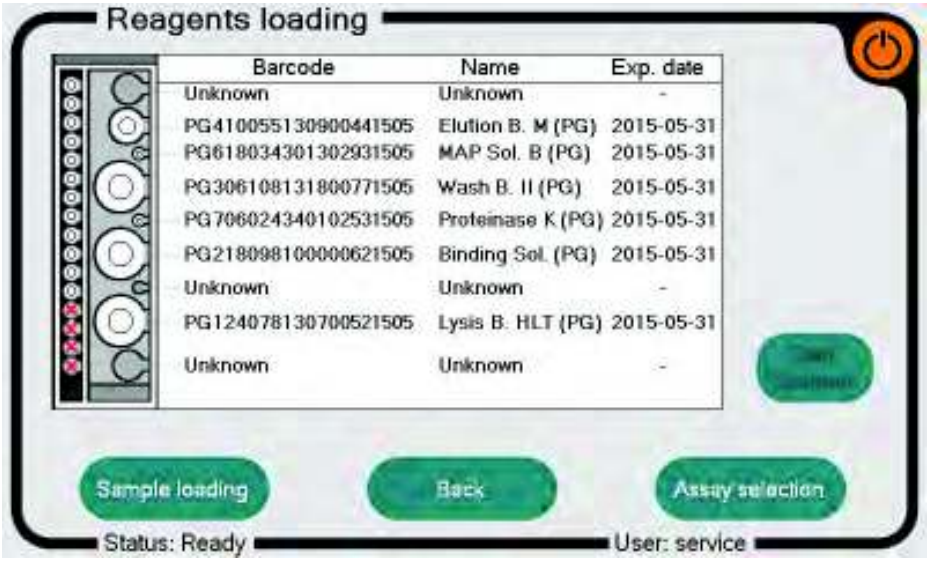

Figure 6: “Reagent-loading” screen of the InviGenius® software

Decap all reagents before inserting them into the reagent rack. Ensure that the bar code labels face to the right side of the loading bay. The order of inserted reagents with the same bottle size is not important because the type and position of each reagent is identified by the unique reagent bar code and the bar code at the corresponding loading position of the reagent rack. However, the possible loading positions are limited by the size of useable bottles.

After rack insertion, the loading status of the reagents is shown. In case of unsuccessful reagent identification, remove the rack, check the bar code orientation and repeat the loading process slowly.

Assay Selection

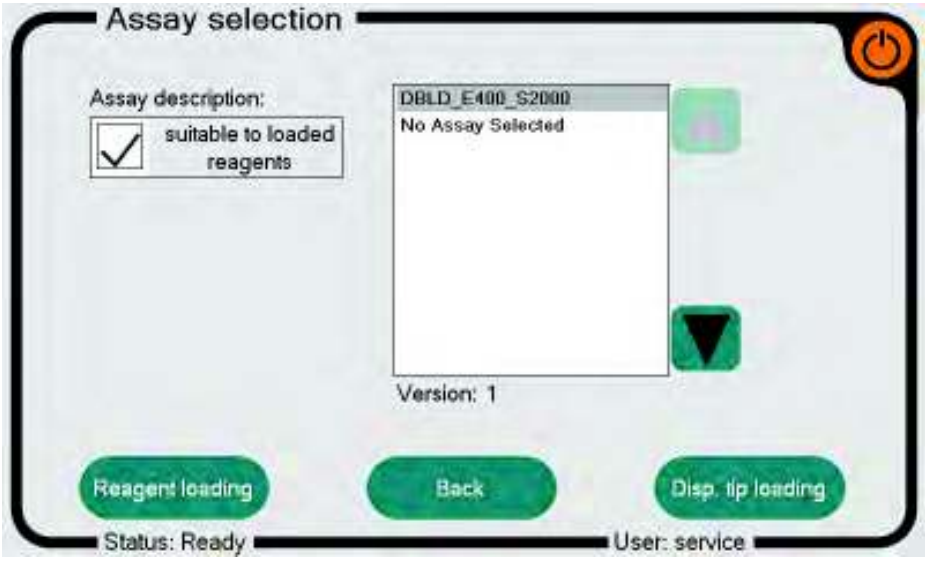

Figure 7: "Assay Selection" screen of the InviGenius® software

Select the assay file "DLBD_E400_S2000" and ensure that the field "suitable to loaded reagents" is checked in order to list only assay files that are compatible with the loaded reagents. If no assay is visible in the list, there is either an error related to reagent loading (blocked barcode label) or at least one reagent container has been loaded that was already in use and does not contain enough reagent to run with the selected amount of loaded samples. If the assay selection was successful, proceed with disposable tip loading.

Disposable Tip Loading:

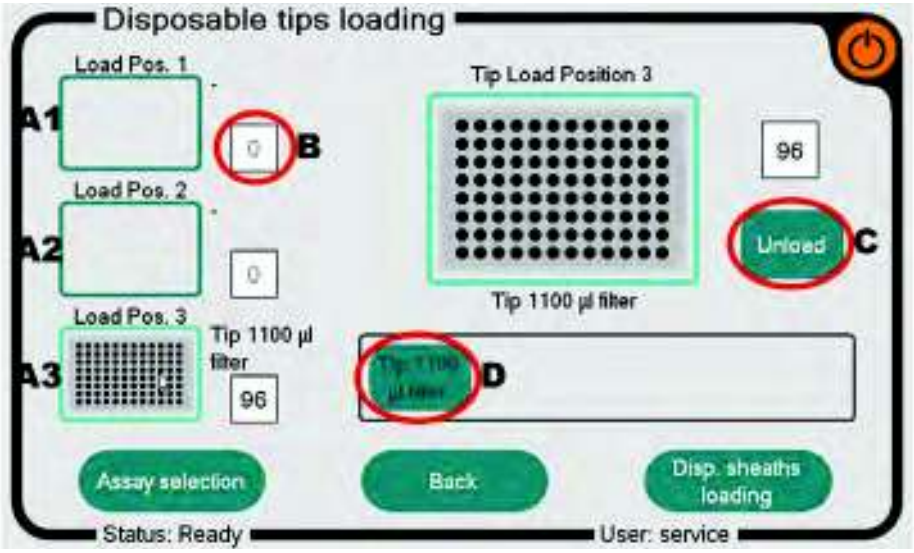

Figure 8, Disposable tip loading screen

There are three tip rack positions available in the InviGenius® system (Fig. 8, A1-A3) that can be loaded with 1100 µl conductive filtered tips to guarantee efficient prevention of cross-contaminations of samples and reagents.

Remaining tip numbers in the rack are shown in field (B). Tip numbers can be changed by pressing the number field (B) and manually enter the number of available tips.

Empty tip racks can be unloaded and reloaded by:

1.) Press the “Loading Position” directly (The software will focus the loading position on the main screen)

2.) Press the Unload Button (C)

3.) The loading position can then be refilled with a new tip rack by pressing on the corresponding tip rack and then selecting the tip type (D).

Note: ***Disposable tips are not supplied within the kit. We recommend the use of validated conductive tips, which can be ordered at STRATEC Molecular. Offered tips are 50 µl conductive tips (10x 96 pieces, order no. 5011120100) and 1100 µl conductive tips (10x 96 pieces, order no. 5011120200). Ensure that conductive tips are used otherwise the liquid level detection (LLD) will not work which will lead to an abort of the run! Due to the installed tip allocation module of the pipettor, it is required that only filter tips are used. Non-filter tips will be rejected by the system.***

To avoid contaminations, never wash or reuse consumed tips!

Disposable Sheaths Loading:

The sheaths function as protection devices for the magnetic rods and are picked up automatically during the run.

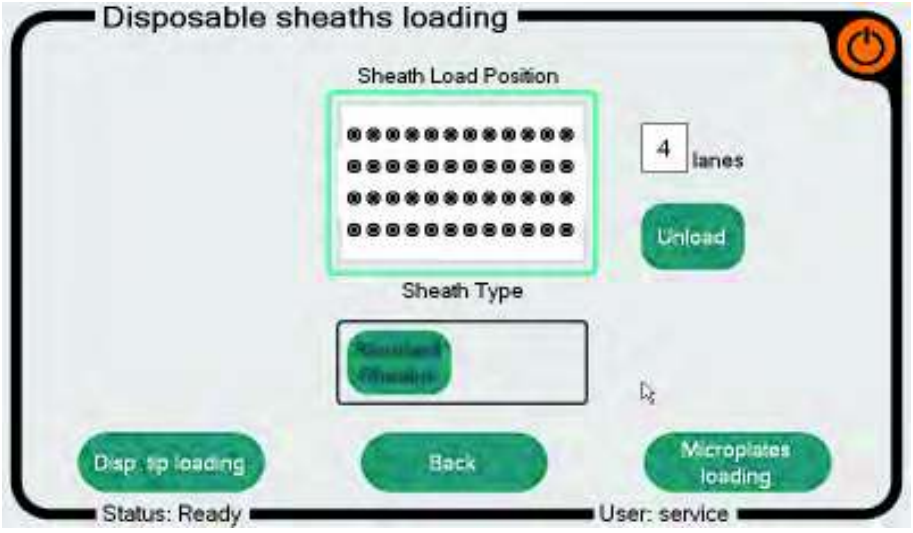

Figure 9, Disposable sheaths loading screen

The loading procedure of the disposable sheaths works analogous to the disposable tip loading screen. For each run, always 12 disposable sheaths (one row of the sheaths rack) are picked up, regardless of the sample number processed. This is done, to assure that all rods are protected against contaminations at all time. In general, the number of sheaths supplied within the kit is sufficient for the amount of runs imprinted on the kit package. In case of low or not enough available sheaths, a reorder at STRATEC Molecular (100 pieces bulk, order nr. 5011120300 or 10x 48 pieces, order no. 5011120400) is possible.

Comparable to the disposable tips loading procedure the number of rows left in the tip rack can be defined by pressing on the number area. Please ensure that the disposable sheaths are loaded (and displayed) consistent to the manually entered sheaths rows in the rack to guarantee correct sheaths pick up. Don’t remove single disposable sheaths within a row of the sheaths rack if less than 12 samples are processed within one run. There is a sheaths detection sensor installed in the instrument. If less than 12 sheaths are picked an error message is displayed and all picked up sheaths will be discarded into the waste container before the next row of sheaths are picked up for verification.

To avoid contaminations, never wash or reuse consumed sheaths!

Plate Loading:

Analogous to the previous loading screens, the incubation, working and elution plates are loaded within the plate loading screen (Figure 10).

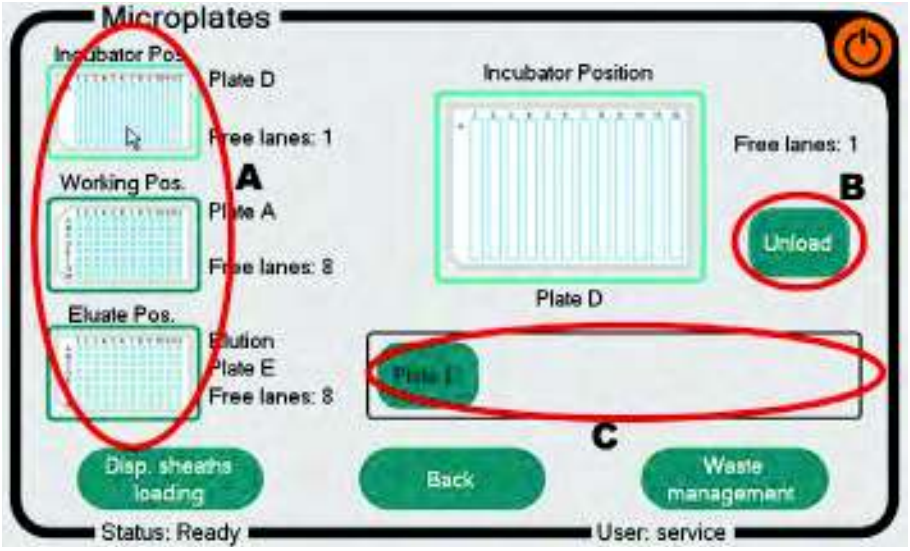

Figure 10, Plate loading screen

In general, an Incubation Plate D (Nerbe Plus 12 well reservoir DWP) is used at the incubator position, a Working Plate A (2 ml Sarstedt DWP) is used at the working position and an Elution Plate E (SLG stripes) is used at the elution position.

Plates can be unloaded / reloaded by:

1.) Pressing the plate position directly (A). The software will focus the plate position on the main screen.

2.) Pressing the “Unload” button (B)

3.) The plate can be reloaded by pressing on the offered plate(s) in (C).

Attention: ***Be aware that it is not possible to manually enter used rows of a plate within the system software. If a partially filled plate is removed from within the system software, all used lanes of that plate will be deleted from the system memory!***

One run with the DNA Blood Maxi Kit on the InviGenius®, using 12 samples, will require one new Incubation Plate D at the incubator position, one new Working Plate A at the working position and one free row within the Elution Plate E located at the eluate position.

Please ensure that the depicted lanes displayed on the monitor are consistent with the real lanes in the corresponding positions.

To avoid contaminations, never wash or reuse consumed plates!

Waste management

Ensure that the waste tray capacity is sufficient for the planned assay. If not, empty the solid waste container. Handle the generated waste accordingly as required by local disposal laws.

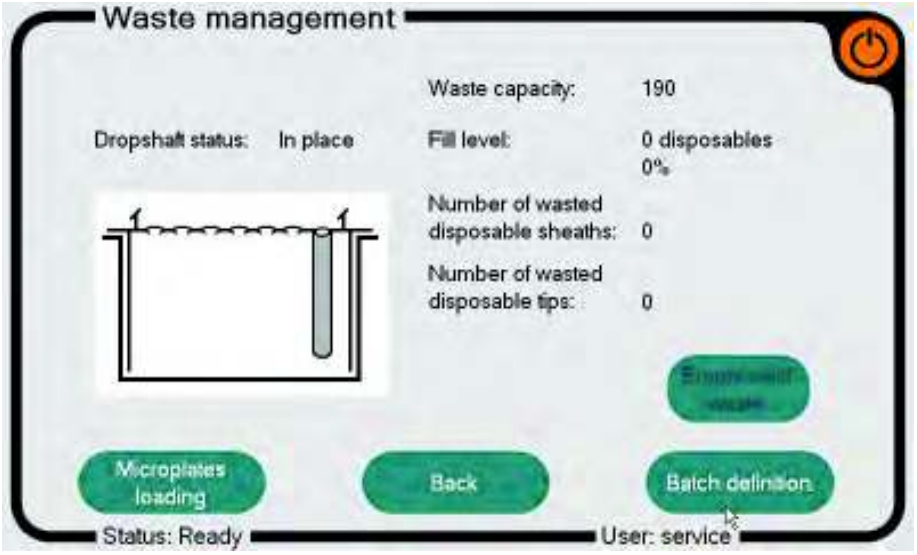

Figure 11; Waste-management-screen

After the waste container was emptied, click on “Empty solid waste” to reset the filling level status.

Batch definition

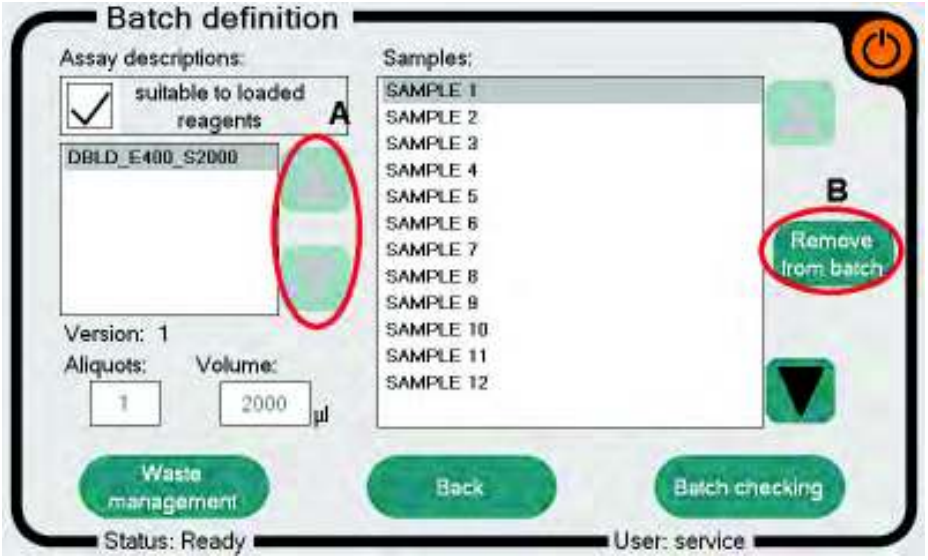

Figure 12; Batch-definition-screen

This screen allows de-/selection of the desired assay and sample numbers in one informational screen. The assay can be switched by using the two arrow buttons (A).

If samples have to be excluded from the batch, use the arrows (B) to switch between samples and click on the “Remove from batch” button (field B). If deselected samples have to be added to the process list, the button “Remove from batch” will be changed to “Add to batch”.

By default, all loaded and recognized samples are selected.

Batch checking

This screen shows a summary of all loaded disposables, samples and buffers in one screen and functions as a final security check. In case of any error, the problem/position is highlighted in red color. To clear a displayed error, click directly on the red highlighted field and follow the instructions printed on the instrument screen.

If no error is displayed proceed by pressing the button “Batch processing”.

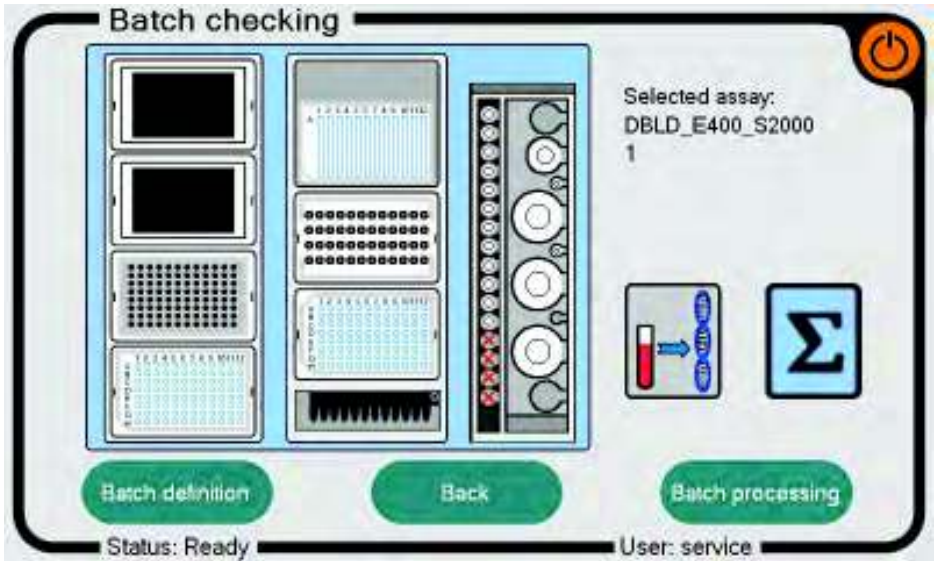

Figure 13; Batch-definition-screen

Batch processing

After the instrument door has been closed, the run can be started by pressing the “Start”-Button (A). The door will be locked during the complete run. The door will only be unlocked after a run has been successfully finished or an error occur that demands user interaction. Do not try to force open the door during a run or the run will be aborted.

If there is an error, the “Start” button is blocked. The user will not be able to start the instrument until the error has been solved. To do so, go back to Batch definition and solve the error (see chapter “Batch definition” for detailed information).

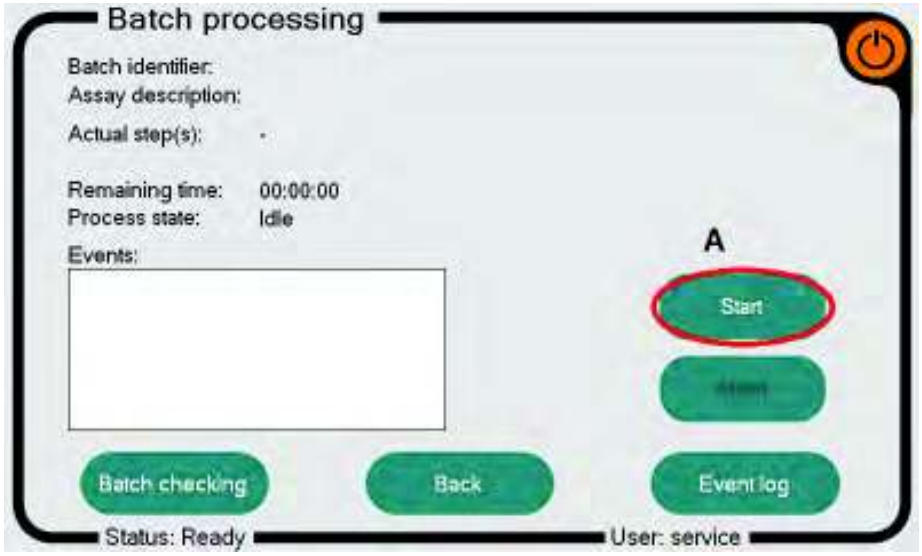

Figure 14; Batch-processing-screen

At the end of the process, the nucleic acid containing eluates are located in the appropriate eluate position and can be used for any further downstream application.

Note: ***The run duration of the Blood Maxi DNA assay, using 12 samples, is approximately 200 minutes.***

After a run

After the run is completed and no additional run should be started, remove all plates and reagents from the instrument and store them accordingly to GLP guidelines. Please keep in mind, that the plates could contain possibly infectious material.

Important: Never erase not completely filled plates from within the software if the plates shall be used for another run. It is not possible to register partially used plates within the software. An erased plate will lead to loss of all stored information.

As with all medical/clinical and diagnostically equipment all waste products (liquids, tips, sheaths and plates) should be treated as potentially dangerous bio-hazard waste.

Daily maintenance (UV decontamination)

The InviGenius® system is equipped with an internal UV lamp (254 nm wavelength) that should be used daily either at the end of the working day or in the morning before a run is started. The suggested decontamination time is about 20 min. To start the UV decontamination go to the main menu of the InviGenius software and select “Maintenance”.

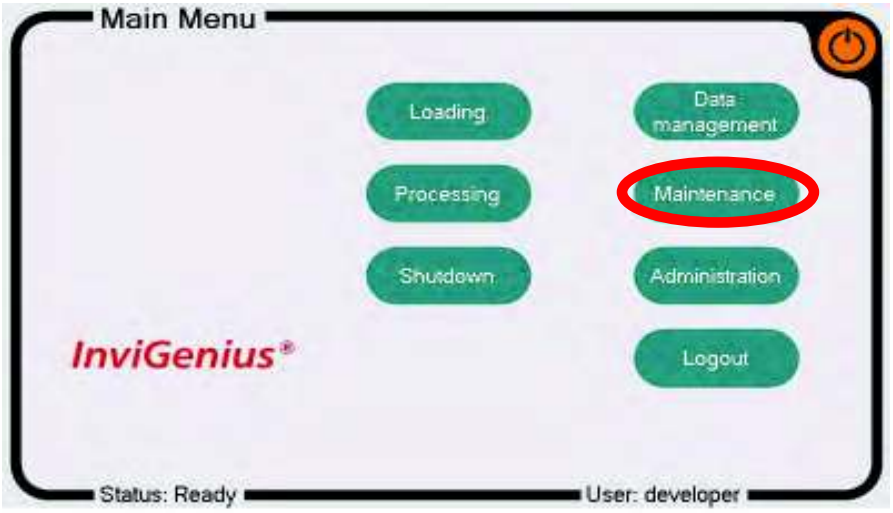

Figure 15: Main screen of the InviGenius® software

When the sub item “Maintenance” is opened, select “UV decontamination”

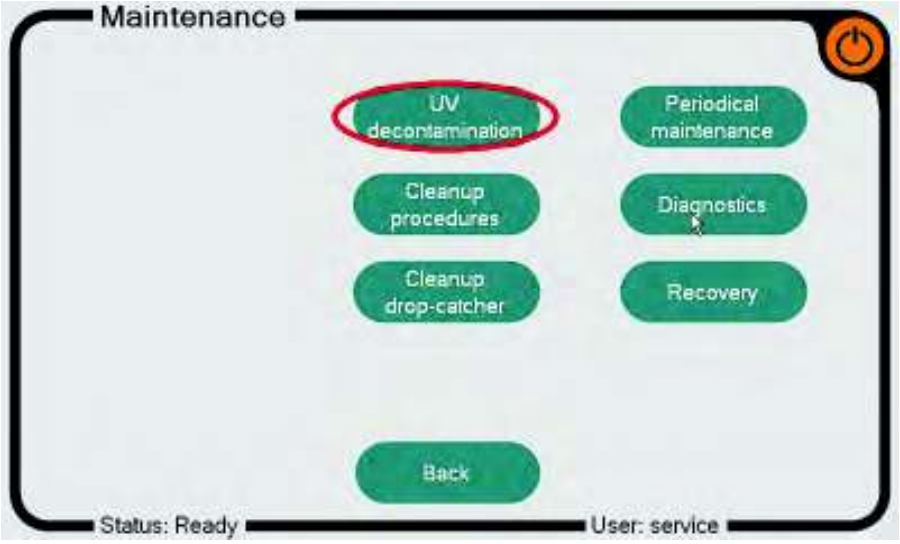

Figure 16: Maintenance screen of the InviGenius® software

In the UV decontamination menu adjust the exposure time (A) and finally press the “Start” button (B). During the decontamination process the instrument door will be locked to prevent any UV radiation release in the lab.

Warning: ***UV radiation is harmful. It causes serious burns of the skin and leads to irreparable damage of the eyes and skin. Ensure that no lab personnel is submitted to direct UV light. Do not try to force open the instrument door during the decontamination process.***

Figure 17: UV decontamination screen

When the decontamination is finished, go back to the main menu by using the “Back” button. The device is now decontaminated and can be either switched off or used for sample processing. We recommend decontaminating the instrument daily.

General notes on handling DNA

Nature of DNA

The length and delicate physical nature of DNA requires careful handling to avoid damage due to shearing and/or enzymatic degradation. Other conditions that affect the integrity and stability of DNA include acidic and alkaline environments, high temperature or UV irradiation. Careful isolation and handling of high molecular weight DNA is necessary to ensure its functionality in various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting, long-template PCR and construction of cosmid libraries.

Handling fresh and stored material before the extraction of DNA

For the isolation of genomic DNA use either fresh or frozen samples (-20°C). This procedure minimizes degradation of crude DNA by limiting the activity of endogenous nucleases.

Storage of DNA

Store the genomic DNA at 2-8°C or -20°C. Keep in mind that the storage of genomic DNA at -20°C may cause shearing, particularly if the DNA is exposed to repeated freezing and thawing cycles. Plasmid DNA and other small circular DNAs can be stored at 2-8°C or at -20°C without risk.

Troubleshooting

Problem	Probable cause	Comments and suggestions
Pipetting distribution errors	Transfer of **Proteinase K** failed	Ensure that lyophilized **Proteinase K** is resuspended with the appropriate volume of water
	Samples transfer failed	The sample tube must contain at least 2.5 ml sample
	Reagent / buffer transfer failed / incomplete	Ensure that the **Wash Buffer** and **Binding Buffer** is filled up properly with ethanol/isopropanol Do not reuse bottles more often than described in Tab.1
Low concentration of extracted DNA	Blood components settled	In case of large sample volumes (>>2 ml) carefully premix the sample tube before inserting it into the sample rack
	No / too much ethanol / isopropanol added to **Wash Buffer / Binding Buffer**	Assure that the **Wash Buffer** / **Binding Buffer** is filled up with ethanol / isopropanol properly as indicated in Tab. 1
Degraded or sheared DNA	Incorrect storage of starting material	Ensure that the storage condition of the starting material was correct Avoid multiple freezing and thawing cycles of the sample
	Old material	Ensure that the starting material is fresh or stored at appropriate conditions (for long-term storage at -20°C) Old material often contains degraded DNA
No assay selectable	Combination of reagents from different kits or blocked barcode during reagent loading procedure	Assure that only reagents belonging to one kit type are used. a combination of reagents belonging to different kit types is not supported by the system Ensure that the reagent barcode label is visible within the reagent rack window
Eluted DNA is brownish colored	Small part of the magnetic particles are left in the elution	Centrifuge the eluates at full speed for 1 min and transfer supernatant to a new plate / tube

Ordering information

Product	Package size	Catalogue No.
InviMag® Blood DNA Maxi Kit/ IG	8 x 12 preps	2431320100

Related products

InviMag® Blood DNA Mini Kit/ IG	8 x 12 preps	2431120100
InviMag® Blood DNA Giga Kit/ IG	8 x 12 preps	2431320400
Invisorb® Spin Blood Mini Kit	50 preparations	1031100200
Invisorb® Spin Blood Mini Kit	250 preparations	1031100300
Invisorb® Spin Blood Midi Kit	50 preparations	1031110300
Invisorb® Spin Blood Midi Kit	250 preparations	1031110500

InviGenius® and consumables

InviGenius®	1 unit	5011100000
Starting Box I/ IG Sheath Box Conductive filter tips, 1 ml; 2 x 2 rack/ pack (384 pieces) 5 Waste Trays 120 sample tubes	1 box	2400110100
Sheath Bundle	10 x 48 pieces	5011100300
Sheaths	1000 pieces	5011100200
Conductive filter tips, 1 ml	10 x 96 pieces	5011100400
Waste tray/ IG	25 pieces	5011100100

Possible suppliers for Isopropanol

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

Sigma
2-Propanol
Order no. 59304-1L-F

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